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Probing live cells with optically driven and monitored micro-rotors

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Abstract

Optically trapped particles can be used as probes to study the mechanical properties of substances on a microscopic scale. Such experiments have been performed on colloids, single bio-molecules such as DNA and proteins, and components of living cells.
A particularly promising type of probe particle is the micro-rotor—an optically trapped and powered microscopic rotating particle. Such a probe allows steady-state motion, and is ideal for the measurement of viscosity on a microscopic scale. The change in polarisation of the trapping beam due to scattering by the probe particle can be used to measure the optical torque acting on, and the rotation of, the probe particle.
We present results from experiments showing that it is possible to rotate small calcite crystals adhering to the membrane of a cell *in vitro*, and measure the applied torque and rotation speed.

1 Introduction

Optical trapping is a powerful tool for the study of physical, chemical, and biological processes on a microscopic scale [1, 2].

Optical forces result from the transfer of momentum to the trapped particle as it scatters the incident trapping beam [3]. As light can also carry angular momentum, the transfer of angular momentum can be used to generate optical torques. The angular momentum carried by a Gaussian laser beam depends on its polarisation state, so processes that change the polarisation state of the beam will result in the production of optical torque. This can be done by trapping a microscopic birefringent particle, which will act as a wave-plate within the beam. Such a particle can be either aligned or continuously rotated [4]. As this process does not involve absorption, with resultant heating, it is suitable for use as a rotating probe for the study of biological samples, including living cells.

The torque acting on the birefringent particle is dependent on the polarisation state of the trapping beam before and after being scattered by the particle. Since the polarisation of the incident beam is typically known, the optical torque can be determined by measuring the polarisation state of the forward scattered light [5]. The optical torque is both maximal and uniform if the incident beam is circularly polarised.

If the probe particle rotates freely, its motion will very rapidly reach steady-state, and can be used to determine the non-optical drag torque due to its surroundings. As the polarisation state of the forward scattered light depends on the orientation of the particle, the rotation rate is measured simultaneously along with the torque [5].

Thus, optically rotated probe particles make suitable probes for the study of the mechanical properties of living biological specimens. Since such probe particles will (at least initially) be outside the cells to be studied, the natural starting point is to investigate the properties of the cell membrane. For some time, optical tweezers have been used in studies of the physical and biological properties of cell membrane [6, 7, 8, 9]; further information could be obtained from studying the responses of cells to the application of controlled torques. An improved knowledge of the mechanical properties of cell membrane is fundamental to the understanding of cell motility and differentiation, as well as the action of drugs and pathogens.

2 Probing cell membrane

The cells to be studied are a culture of 3T3 cells, spread on a microscope cover slip. The microscopic birefringent probe particles are produced by grinding Iceland spar (calcite) in a phosphate buffer solution, and these are added to the culture. After the addition of a microscope slide, the sample is placed into an optical tweezers apparatus built into an inverted microscope as shown in figure 1.

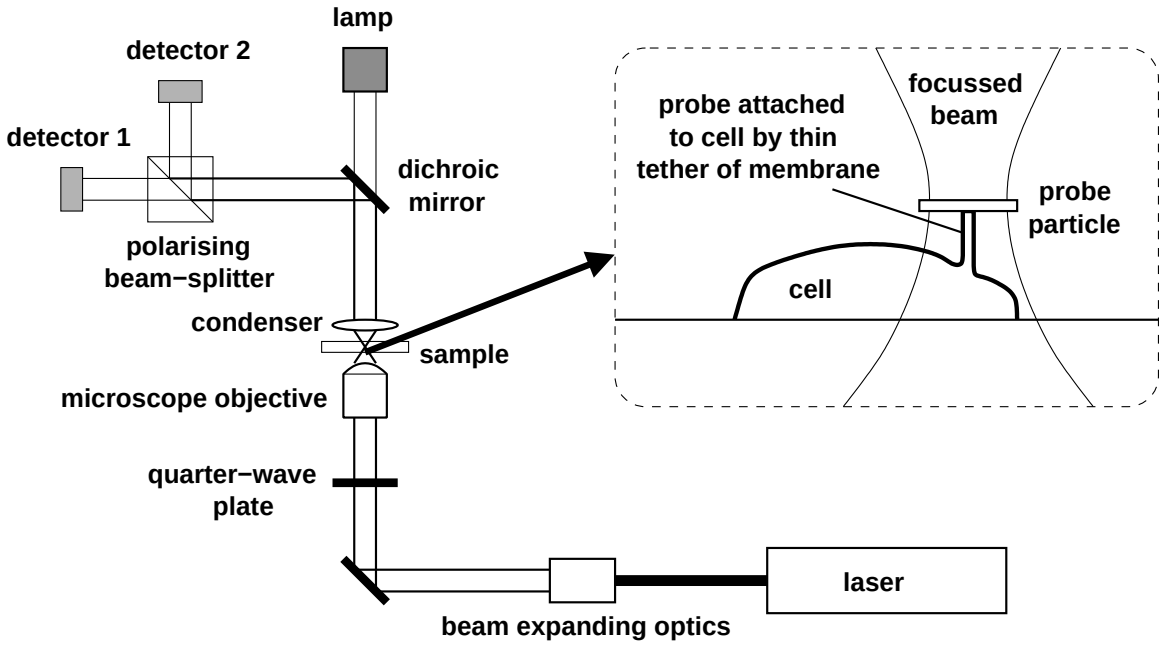

Figure 1: An experiment to measure the mechanical properties of cell membrane.

The optical tweezers consists of an expanded beam from a Nd:YAG laser directed upwards into the back of the microscope objective, which tightly focusses the beam (as required for trapping). A quarter-wave plate is used to produce a circularly polarised trapping beam.

Calcite particles adhering to the cell membrane could be rotated in this apparatus. The rotation and optical torque were measured by using the microscope illumination collimator to collect the forward scattered light, which is then separated using a high-reflectance dichroic beam splitter. A polarising beam splitter was then used to separate the two linearly polarised components, and the power in

each component measured.

If the probe particle is a flat disc, rotating at an angular speed Ω, and illuminated by a circularly polarised plane wave, the observed power of one polarisation component P_{m} is [5]

$$P_{\mathrm{m}} = \{1 + (1 - |\sigma_{\mathrm{zout}}|)\cos 2\Omega t\}P/2 \tag{1}$$

where P is the total beam power, and σ_{zout} is the degree of circular polarisation of the forward scattered light.

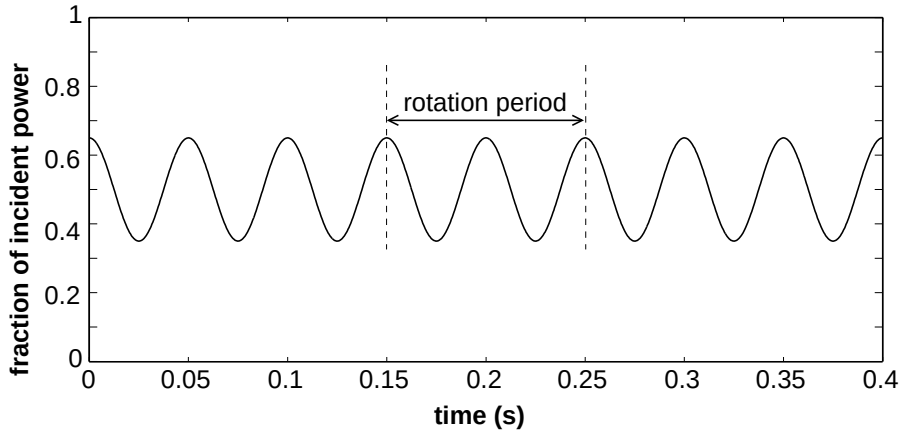

Figure 2: Ideal theoretical measured power for a probe particle rotating at 10 Hz. $\sigma_{\mathrm{zout}} = +0.7$

From the measured power curve, we can determine the total beam power, the rotation speed, and the torque, which is given by [5]

$$\tau = (1 - \sigma_{\mathrm{zout}})P/\omega \tag{2}$$

where ω is the optical angular frequency.

3 Results

A typical curve for the measured power of a single polarisation component, and the corresponding spectral power density is shown in figure 3.

The resemblance between the observed curve (fig 3) and the ideal curve (fig 2) is clear. Both the optical torque and rotation speed can be found.

The differences between observed curves and ideal theoretical curves vary with the particular particle and cells used; the probe particle is in an complex environment and often does not rotate smoothly. Additionally, if the probe particle is uneven, and not centred on the beam axis, an additional orientation dependence is introduced.

In these experiments, the probe particles were attached to tubular protuberances from the cells. As the particles rotate continuously, without coming to a stop due to the tube becoming fully twisted, slip must be occurring. The calcite crystals could often be seen to rotate a few turns in the opposite direction when the optical torque was removed, clearly showing that the tube remained intact, but only needed a small number of turns to unwind after a very large number of optically driven rotations. With a power of 100 mW, rotation rates in excess of 200 Hz were recorded, without the calcite particle separating from the membrane.

These experiments show that it is possible to rotate calcite probe particles adhering to the membrane of a cell *in vitro*, and to measure the applied torque, both by purely optical means.

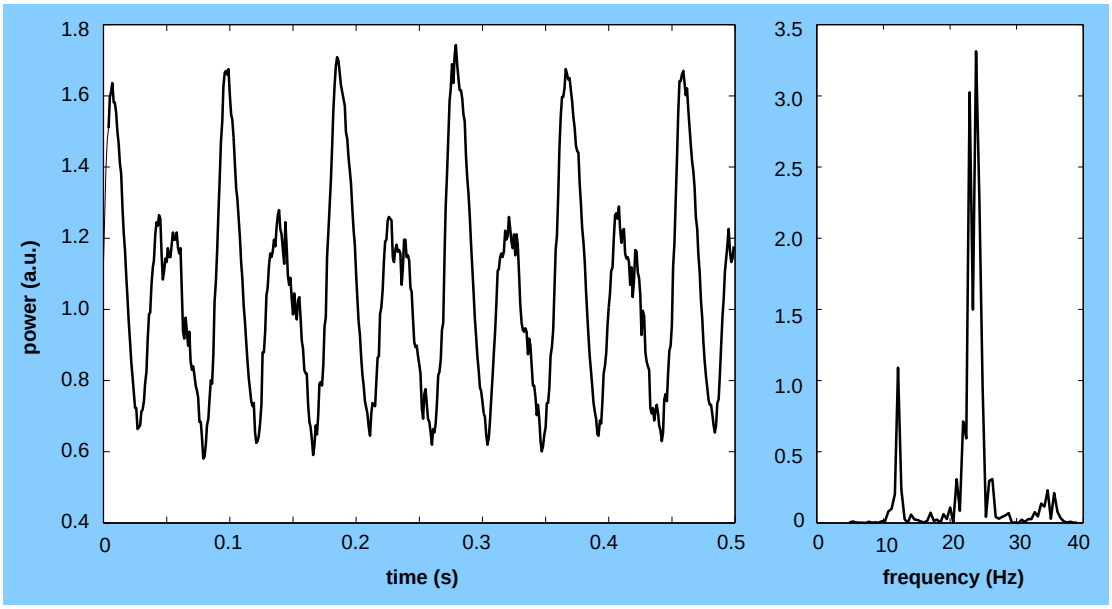

Figure 3: Measured power of one linear polarisation component, for a probe particle trapped by a 150 mW beam with $\lambda_0 = 1064$ nm. The rotation rate is 12.2 Hz and the optical torque is 32 pN·μm.

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